Kidney International, Vol. 46 (1994), pp. 986–992

Effects of complement activation products on the synthesis of decay accelerating factor and membrane cofactor protein by human mesangial cells

FERNANDO G. COSIO, TORU SHIBATA, BRAD H. ROVIN, and DANIEL J. BIRMINGHAM

Department of Internal Medicine, Nephrology, The Ohio State University, Columbus, Ohio, USA

Effects of complement activation products on the synthesis of decay accelerating factor and membrane cofactor protein by human mesangial cells. We previously demonstrated that activation of terminal complement components (C8 and/or C9) increases the synthesis and expression of decay accelerating factor (DAF) on human glomerular cells. DAF is a cell membrane-associated complement regulatory protein that inhibits complement activation on cell surfaces. In the present studies we evaluated, first, the mechanisms by which complement activation stimulates DAF synthesis, and second, the effect of complement activation on the synthesis, and expression of membrane cofactor protein (MCP), another complement regulatory protein, by human mesangial cells (HMC) in culture. Complement activation by immune complexes resulted in increased DAF mRNA levels by at least two mechanisms: deposition of activated C3 on HMC and generation of soluble complement activation products, specifically C5a. The increase in DAF mRNA levels induced by activated C3 or C5a was short lived (less than 4 hr). In contrast, the up-regulation of DAF mRNA levels induced by activation of the complete complement cascade persisted for at least eight hours. The effect of complement activation on DAF mRNA levels was not affected by cycloheximide, a protein synthesis inhibitor. However, cycloheximide alone resulted in a significant up-regulation of DAF mRNA levels on HMC. In contrast to those findings, complement activation did not cause an up-regulation of MCP mRNA, nor an increase in the synthesis of this protein. However, by FACS, complement produced a small but significant increase of MCP protein levels on HMC. In conclusion, both MCP and DAF are present on HMC. Several activated complement components are capable of increasing DAF mRNA levels, but DAF protein levels increase only after activation of the whole complement cascade. Complement activation has no effects on the synthesis of MCP.

Complement activation on cell surfaces is inhibited by a series of cell-associated complement regulatory proteins (C′RP) [1, 2], and other components of the cell surface [3]. Cell associated C′RP protect cells against complement mediated lysis [1] by inhibiting complement activation either at the level of C3/C5 (C3-C′RP) or C8/C9. Several characteristics of this protective function are of interest. First, cytoprotection by C′RP has at least partial homologous restriction, that is, C′RP provide protection preferentially against complement components of the same species as the cell [1, 2, 4, 5]. Second, in most cells, protection against complement mediated lysis is provided by the combined effects of several C′RP [6–9]. Consistent with these observations, patients with congenital deficiencies of several C′RP have more severe episodes of hemolysis than patients with more selective deficiencies [8, 10]. Finally, more recent experiments have demonstrated that the relative protective effects of each C′RP varies from cell to cell. For example, in some cells, cytoprotection against complement is provided mainly by DAF while in other cells protection is provided mainly by MCP [11].

Most forms of glomerular disease in humans involve immune complex (IC) formation and complement activation. Thus, factors, such as C′RP, that may protect glomerular cells against complement mediated effects may influence the extent of tissue damage that occurs during glomerulonephritis. Recently, we investigated the mechanisms involved in the protection of human glomerular cells from complement effects. In initial studies we and others evaluated the presence of C3-C′RP in normal and diseased human kidneys. All three known cell associated C3-C′RP, that is, complement receptors type 1 (CR1), decay accelerating factor (DAF), and membrane cofactor protein (MCP), are present in the human kidney. CR1 is present exclusively on glomerular epithelial cells [12]. DAF is present at low levels on glomerular cells from normal kidneys and at higher levels in cells of the juxtaglomerular apparatus [13]. In contrast, kidneys from patients with complement mediated glomerulonephritis demonstrated increased DAF expression in glomeruli, interstitium and blood vessels [13]. Finally, MCP is present at high levels on renal tubular epithelial cells and at lower levels in mesangial and endothelial cells. We have not detected significant differences between the MCP staining of normal and diseased kidneys [14]. These findings suggest that C3-C′RP may have a role in protecting kidney cells from complement effects. In addition, during disease, glomerular cells are induced to express more DAF and this effect may provide additional protection against complement effects.

In subsequent studies, we have demonstrated that complement activation increases DAF expression on the membrane of HMC *in vitro* [15]. Furthermore, we have shown that: (1) The effect of complement on DAF expression requires activation of terminal complement components including C8 and/or C9. Specifically, human serum deficient in C5 or C8 and soluble activated complement components had no significant effects on DAF expression. (2) The increased expression of DAF on HMC exposed to complement requires new RNA and protein synthesis. Based on

Received for publication October 20, 1993
and in revised form April 29, 1994
Accepted for publication May 6, 1994

these studies we postulated that complement activation increases DAF gene transcription and DAF mRNA translation resulting in increased cell membrane DAF expression. This postulate was tested in the experiments described in the present manuscript.

Methods

Preparation of mesangial cell cultures and exposure to complement activation products

Human mesangial cell (HMC) cultures were derived from kidneys deemed unsuitable for transplantation. Cells used in the present experiments were derived from five different adult kidneys. We have previously described the methods used for the isolation and characterization of HMC [15, 16]. In brief, renal cortex was minced and passed through progressively smaller-sized sieves (Bellco Glass Inc., Vineland, New Jersey, USA). Glomeruli, retained by the last sieve, were treated with 0.1% collagenase (Worthington Biochemical, Freehold, New Jersey, USA) and cultured in plastic culture flasks (Corning Glass Works, Corning, New York, USA) with media 199 (Gibco, Grand Island, New York, USA) containing 10% Nu-serum (Collaborative Research, Bedford, Massachusetts, USA) and antibiotics. Cellular outgrowths of the glomeruli were passed into new flasks and characterized as described [15, 16].

To expose HMC to immune complex (IC) formation and complement activation we used previously described methods [15]. Briefly, confluent HMC layers were incubated first with phenilated gelatin (DNP-GL), an antigen that binds avidly to fibronectin present in the matrix produced by HMC in culture. After removing the unbound DNP-GL, HMC were incubated with rabbit IgG anti-DNP antibodies in the presence of 1:40 diluted AB+ human serum (HS) or HS deficient in specific complement components (Quidel, San Diego, California). The dilutions of complement deficient HS used in these experiments were such that, once supplemented with the missing complement component (Quidel), these sera produced the same degree of hemolysis of antibody sensitized sheep erythrocytes as 1:40 diluted whole HS [15]. In control experiments, HMC were incubated with: (1) DNP-GL and HS; (2) DNP-GL in the presence of normal rabbit IgG (Sigma) and HS; (3) rabbit anti-DNP antibodies and HS; and (4) DNP-GL and anti-DNP antibodies in the absence of HS. Unless otherwise indicated, these incubations were continued for four hours.

In some experiments HMC were incubated with media or IC and HS in the presence of cycloheximide (10 μg/ml) [15], a protein synthesis inhibitor. Cycloheximide was added to the cells together with the anti-DNP antibodies and HS and the incubation was continued for four hours.

To test the effects of soluble activated complement components, HMC were incubated with zymosan activated serum (ZAS) [15] for four hours. Prior to addition to the culture all ZA sera were heat inactivated at 56°C for 30 minutes. In other experiments, HMC were incubated with recombinant C5a (rC5a) (Sigma) suspended in media with 0.25% bovine serum albumin (BSA). Control cells, in these experiments, were incubated for the same period of time with 0.25% BSA.

The effects of complement on C3-C'RP synthesis might be due to the binding of activated complement components to previously unrecognized complement receptors on HMC. To test functionally for the presence of such putative receptors, HMC were incubated for four hours with zymosan particles coated with activated complement components. To prepare the complement coated particles, zymosan particles (5 mg/ml) were incubated with HS or heat inactivated serum (HIS) (both diluted 1:20) for 30 minutes at 37°C. Complement coated zymosan particles were washed three times with sterile culture media and then added to HMC flasks (5 mg/flask).

Isolation of HMC RNA and Northern blot analysis of DAF and MCP messenger RNA (mRNA)

HMC were cultured for 7 to 10 days in 150 cm^2 flasks and then exposed to IC formation and complement activation products as described above. At the end of the incubation, HMC were detached from the culture flask by incubation with 0.25% Trypsin/0.56 mM EDTA (Gibco) for 10 minutes and washed with sterile media. HMC RNA was isolated following the RNAzol method (Cinna/Biotex Laboratories International, Inc., Friendswood, Texas, USA) described previously [17, 18]. Total RNA concentration was measured by light absorption at 260 nm. For Northern blot analysis [18], 15 to 25 μg of RNA was loaded in each lane of a 1% agarose gel and the RNA was separated by electrophoresis. RNA was transferred to nylon membranes (Hybond-N, Amersham) by capillary action. DAF and MCP mRNAs were identified by hybridization of nylon membranes with ^{32}P labeled DAF cDNA [19] and MCP cDNA [20] (gifts from Dr. J.P. Atkinson, Washington University, St. Louis, Missouri, USA). Following autoradiography, the amount of DAF mRNA was quantitated by densitometry (Pharmacia LKB. UltraScan L). The amount of total RNA in each lane of the gel was quantitated by densitometry of 18s and 28s ribosomal RNA bands stained with ethidium bromide (EB). To correct for variations in RNA loading, densitometry values for DAF mRNA were divided by densitometry values of total RNA (18s band) in the same lane. Changes in the amount of DAF mRNA in stimulated MC are expressed as percent of the DAF mRNA in MC incubated under control conditions.

Quantitation of DAF and MCP by flow cytometry (FACS)

In these experiments, HMC were incubated under the various conditions described above for four hours. Subsequently, HMC were detached by incubation with Trypsin/EDTA and maintained at 4°C. HMC were incubated with mouse monoclonal anti-DAF antibodies (1A10, a gift from Dr. V. Nussenzweig), mouse monoclonal anti-MCP antibodies (GB24, a gift from Dr. J.P. Atkinson) or irrelevant monoclonal antibodies. After a 30-minute incubation, HMC were washed three times and incubated with FITC-labeled sheep anti-mouse IgG (Zymed) for 30 minutes. At the end of the incubation, HMC were fixed with 1% paraformaldehyde and analyzed by FACS [21]. Both the percent of FITC positive HMC and the relative fluorescence intensity of cells, assessed in a linear scale, were determined. Nonspecific staining was assessed in HMC incubated with irrelevant monoclonal antibodies and FITC labeled secondary antibodies. Nonspecific values were subtracted from values obtained from cells stained with anti-DAF or anti-MCP antibodies to obtain specific values.

Quantitation of HMC MCP by radioimmunoassay

We used the same methods previously described for the quantitation of DAF on HMC [15]. This method of quantitation was used, in addition to FACS, because for the RIA HMC do not need

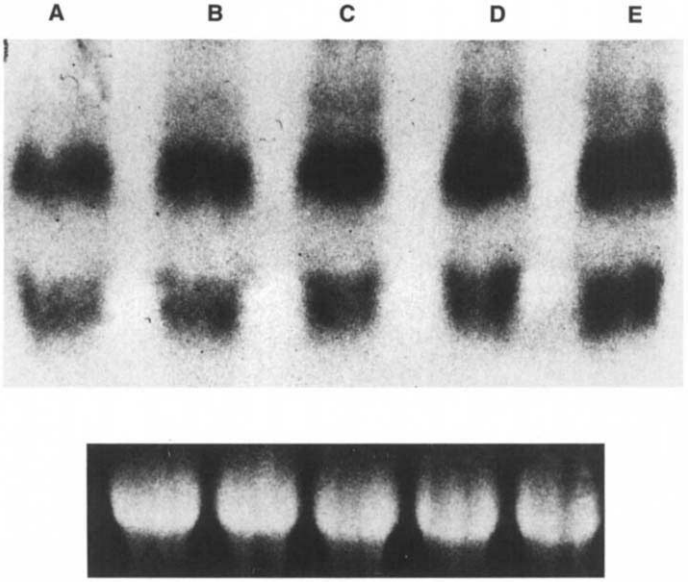


Fig. 1. Top. *DAF mRNA in HMC incubated with media and HIS for 4 hours (lane A), IC and HS for 2 hours (lane B), 4 hours (lane C), 6 hours (lane D) or 8 hours (lane E).* Autoradiograph was developed for 7 days. **Bottom.** EB fluorescence of 18S ribosomal RNA on the same gel demonstrating uniform RNA loading.

to be exposed to trypsin prior to quantitation. Thus, any possible effects of trypsin on MCP are avoided. Briefly, HMC were incubated with media and HS, IC and HIS, IC and HS and IC and HS in the presence of cycloheximide (10 μg/ml). At the end of the incubation, HMC were washed with cold (4°C) media and maintained at that temperature during the following steps: (1) incubation, for 30 minutes, with mouse monoclonal anti-MCP antibodies (GB24, a gift from Dr. J.P. Atkinson) [22, 23], diluted in 3% BSA and 0.05% Tween; (2) after removing the unbound primary antibody, HMC were incubated, for 45 minutes, with ^{125}I labeled rabbit anti-mouse IgG, diluted in the same media as the primary antibody; (3) HMC were then washed, detached from the culture wells by incubation with Trypsin/EDTA and the mixture was counted for radioactivity. Subsequently, HMC were separated from the supernatant by centrifugation and counted in a hemocytometer. Results were expressed as pg of secondary antibody per 10^3 HMC. In each experiment, control cells were incubated first, with irrelevant monoclonal antibodies and then with ^{125}I labeled secondary antibodies [15]. Antibodies were radiolabeled by the Iodo-Gen method (Pierce Chemical Co., Rockford, Illinois, USA).

Analysis of data

Data are expressed throughout the manuscript as mean ± standard error of the mean. Data were analyzed by ANOVA, with a Bonferroni post-*t*-test, or paired *t*-test as indicated in the text.

Results

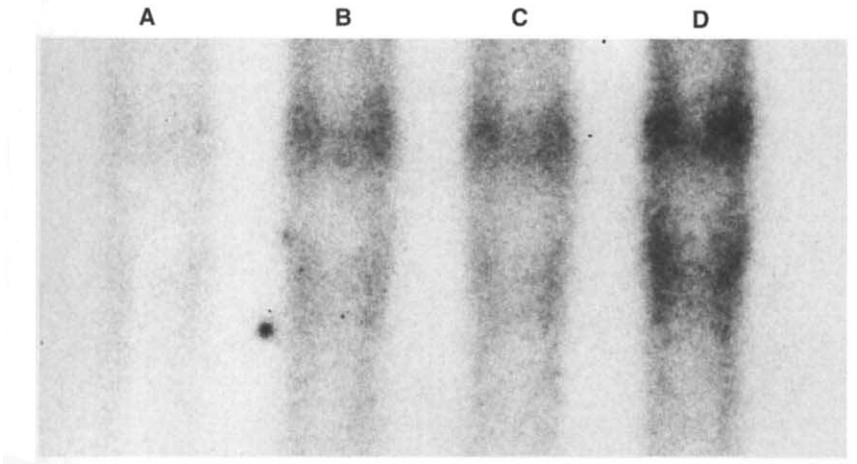
Effects of complement activation on HMC DAF mRNA levels

HMC were exposed to IC formation and complement activation and DAF mRNA was analyzed by Northern blotting techniques. As demonstrated in Figure 1, exposure to complement activation products for two to eight hours was associated with a progressive increase in DAF mRNA levels in HMC. By densitometry of values obtained in this particular experiment, the increase in DAF mRNA 2.2 kb transcript at 2, 4, 6, and 8 hours was 123%, 169%, 202%, and 218% of control values, respectively.

Previous studies have identified at least three different DAF mRNA transcripts of 2.2, 1.6 and 1.5 Kb [24] (Fig. 1). All these transcripts were identified on HMC although in most Northern gels the 1.5 and 1.6 transcript are too close for easy identification. Exposure to complement activation for four hours caused significant increases in both the 2.2 Kb (249 ± 32% of control, $N = 20$) and the 1.5 to 1.6 Kb (244 ± 38%, $N = 20$) DAF mRNA transcripts. In all subsequent experiments, densitometry data is based on analysis of the 2.2 Kb DAF mRNA transcript. In control experiments, we found that compared to HMC incubated with media, HMC DAF mRNA levels were not significantly affected by antigen alone, IC alone or HS alone (data not shown).

To further characterize the effects of complement activation products, HMC were exposed to IC and human serum or IC and human serum deficient in C3, C5 or C8. IC formation in the presence of C3 deficient serum had no significant effects on DAF mRNA levels (98 ± 28%, $N = 5$). In contrast, IC formation in the presence of HS, C5 deficient or C8 deficient serum (Fig. 2) caused significant increases in DAF mRNA levels (213 ± 21, $N = 23$; 178 ± 24%, $N = 7$; 218 ± 43%, $N = 5$, respectively, ANOVA $P < 0.0001$ compared to DAF mRNA levels in unstimulated cells). C5 deficient had a tendency to produce a smaller increase in DAF mRNA levels than whole HS; however, these differences did not reach statistical significance. To further analyze the induction of DAF mRNA by C5 deficient serum, HMC were incubated with IC and C5 deficient serum for variable periods of time (Fig. 3). As can be seen, activation of C5 deficient serum induced a peak increase in DAF mRNA levels after two hours of incubation, and DAF mRNA levels decreased thereafter. Values obtained by densitometry of the 2.2 Kb transcript in this particular Northern included: 1 hour, 94%; 2 hours, 187%; 3 hours, 131%; 6 hours, 117% of control. These findings imply that activation of C3 is required and is sufficient to up-regulate DAF mRNA levels. To assess whether the effects of activated C3 were mediated by previously undetected HMC receptors for C3 products, HMC were incubated with zymosan particles coated with activated complement products. These particles had no significant effects on DAF mRNA levels (DAF mRNA levels, 92 ± 10%, $N = 4$).

To test the effects of soluble activated complement components on DAF mRNA levels, HMC were incubated with zymosan activated sera. ZAS produced a significant up-regulation of DAF mRNA levels (262 ± 108, $N = 5$), although the degree of DAF mRNA up-regulation was quite variable among experiments. To determine which soluble complement component was responsible for the effects of ZAS on DAF mRNA, HMC were incubated with increasing doses of rC5a. The anaphylatoxin rC5a significantly up-regulated DAF mRNA levels and the effects of rC5a were dose dependent with a maximum increase in DAF mRNA levels occurring after incubation with 2 μg/ml (215%) (Fig. 4). Subsequently we showed that, in most experiments, incubation of HMC with rC5a for variable periods of time (Fig. 5) caused a peak induction of DAF mRNA after two hours of incubation followed by a decline in DAF mRNA levels.



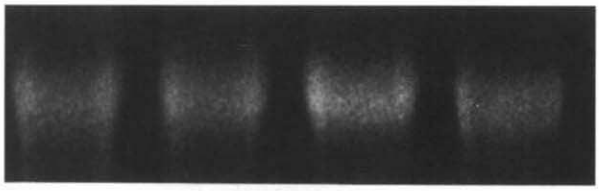
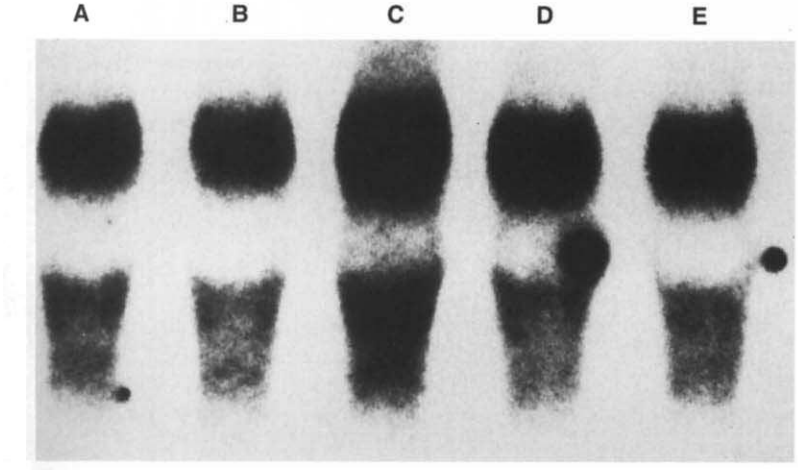


Fig. 2. Top. *DAF mRNA in HMC incubated for 4 hours with media and HS (lane A), IC and HS (lane B), IC and C5 deficient serum (lane C), or IC and C8 deficient serum (lane D).* Autoradiograph exposed for 5 days. **Bottom.** EB fluorescence of 18S ribosomal RNA on the same gel demonstrating uniform RNA loading.



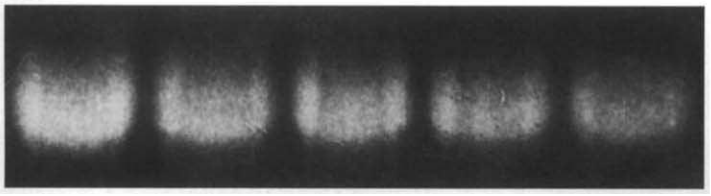


Fig. 3. Top. *Northern blot of DAF mRNA isolated from HMC incubated with IC and C5 deficient serum for B, 1 hour; C, 2 hours; D, 4 hours; E, 6 hours. A, control lane.* Autoradiograph exposed for 2 days. **Bottom.** EB fluorescence of 18S ribosomal RNA on the same gel demonstrating uniform RNA loading.

Role of protein synthesis on DAF transcription

To assess whether the effects of complement activation on DAF mRNA levels required protein synthesis, in the next series of experiments HMC were incubated with media alone or IC and HS in the presence or absence of cycloheximide (10 μg/ml; $N = 4$ for each condition). Compared to control cells incubated with media and HIS, incubation with IC and HS caused an increase in DAF mRNA levels (193 ± 39%). However, the effects of IC and HS were not significantly affected by cycloheximide (207 ± 35%). In addition, cycloheximide alone caused a significant up-regulation

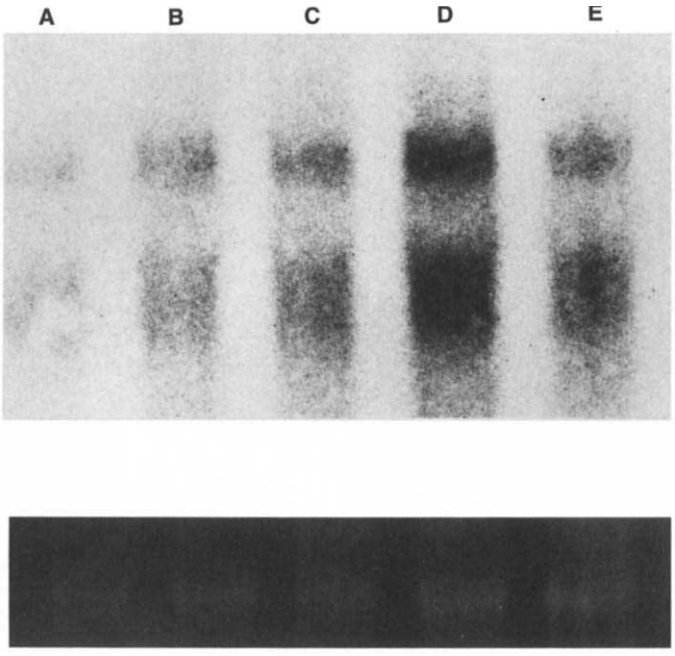


Fig. 4. Top. *Effects of increasing amounts of rC5a on DAF mRNA levels: (A) cell incubated without rC5a; (B) cells incubated for 4 hours with rC5a at 0.5 μg/ml; (C) rC5a at 1 μg/ml; (D) rC5a at 2 μg/ml; (E) rC5a at 4 μg/ml.* **Bottom.** EB fluorescence of 18S ribosomal RNA on the same gel demonstrating uniform RNA loading.

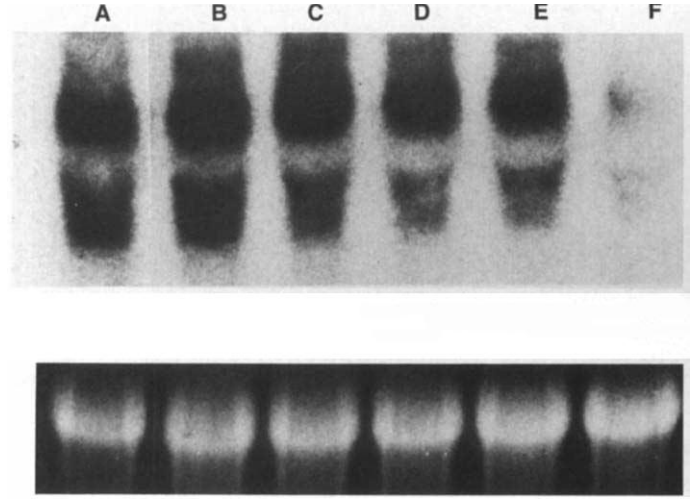


Fig. 5. Top. *Northern blot analysis of DAF mRNA isolated from HMC incubated with rC5a (2 μg/ml) for B, 1 hour; C, 2 hours; D, 4 hours; E, 6 hours.* A, DAF mRNA in control cells incubated with media alone. Autoradiograph exposed for 4 days. **Bottom.** EB fluorescence of 18S ribosomal RNA on the same gel.

of DAF mRNA levels (198 ± 35%), suggesting that in unstimulated HMC, DAF mRNA levels may be suppressed by a control mechanism that requires protein synthesis.

Effects of complement activation on DAF and MCP protein expression on HMC

In these experiments we first used FACS analysis to determine the effect of complement activation on DAF and MCP protein levels on the HMC membrane (Table 1). As can be seen, both DAF and MCP are present on unstimulated HMC. Following complement activation for four hours, the percent of cells positive for DAF and the amount of DAF per cell (fluorescence intensity) are significantly increased. Similarly, MCP levels on HMC are increased after exposure to complement activation products although the changes in MCP are of lesser magnitude than the changes in DAF.

Table 1. FACS analysis of DAF and MCP levels on HMC membranes following exposure to IC formation and complement activation

Parameter	Antigen (N)[a]	Incubation conditions		Paired t (P)
		Media + HS	IC + HS	
Positive cells %	DAF (5)	41 ± 5	62 ± 7	0.006
FITC intensity[b]	DAF (5)	59 ± 14	202 ± 47	0.02
Positive cells %	MCP (3)	77 ± 3	83 ± 3	0.05
FITC intensity	MCP (3)	144 ± 14	282 ± 24	0.05

[a] N represents the number of experiments
[b] FITC intensity was measured by arbitrary fluorescence units

We next assessed MCP levels on HMC by RIA. By ANOVA there were no statistically significant differences in MCP levels in HMC incubated with: (1) HS alone (30.7 ± 5 pg Ab/10^3 cells), (2) IC + HIS (40.8 ± 10 pg Ab/10^3 cells); (3) IC + HS (49.3 ± 10 pg Ab/10^3 cells); and (4) IC + HS in the presence of cycloheximide (41.8 ± 9 pg Ab/10^3 cells) (ANOVA P = 0.4). Thus, the effects of complement activation on HMC MCP are not dependent on protein synthesis. To further assess the effects of complement activation on MCP synthesis, MCP mRNA levels were analyzed on HMC exposed to IC formation and complement activation. Complement activation had no significant effect on MCP mRNA levels (data not shown).

Discussion

In the present study we demonstrated that deposition of activated complement components on HMC increases DAF mRNA levels and, under certain circumstances, increases DAF protein levels on the HMC membrane. In contrast, under the same conditions, complement activation does not increase the synthesis of a second C3-C'RP, MCP.

Complement activation up-regulates DAF mRNA levels by at least two mechanisms: (1) deposition of activated C3 products on HMC, and (2) generation of soluble activated complement products. The evidence for a role of C3 deposition is as follows: IC activation of C5 deficient serum, that is, activation of the classical complement pathway including C3, increased DAF mRNA levels. In contrast, IC activation of C3 deficient serum had no significant effects on DAF mRNA levels, indicating that the effect of C5 deficient serum is due to activation of C3. The effect of activated C3 on DAF mRNA levels suggests that HMC may have the capacity to bind degradation products of C3 generated extrinsically to the cell membrane. However, previous studies [12, 15, 25, 26] and the findings of the present studies provide no evidence for the presence of C3 receptors on HMC. Two other possible mechanisms may explain signal transduction via C3 deposition on cell membranes: (1) We speculate that the binding of activated C3 (C3b) to plasma membrane proteins may generate intracellular signals. Although there is no direct evidence for this effect of

activated C3, previous studies showed that activated complement components, earlier that C7, can cause changes in intracellular mediators such as calcium and cAMP [27]. (2) It has been demonstrated that binding of certain monoclonal antibodies to DAF may generate signal transduction [28]. The participation of these mechanisms in the regulation of DAF mRNA levels by C3 remain speculative.

ZAS caused an up-regulation of DAF mRNA levels and this effect could be accounted for, at least in part, by the effects of C5a on HMC. However, the present studies do not exclude an effect mediated via C3a. These findings may indicate the presence of receptors for C5a on HMC. In addition, a previous study suggest that the binding of analphylatoxins to cells may be charge dependent and not receptor mediated [29]. Similar to the effects of C5a on DAF [15], in other cell systems C5a increases transcription but not translation of other proteins [30]. The doses of rC5a used in these previous experiments is in the range of doses used in the previous study. To our knowledge the presence of C5a receptors on HMC has not been explored. However, injections of C5a into the kidney result in significant changes in glomerular filtration rate perhaps due to mesangial cell contraction [31]. In addition, it has been suggested that C5a may cause hemodynamic alterations in the diseased glomerulus [32].

The present results complement previous findings from our laboratory demonstrating that activation of terminal complement components (C8 and/or C9) induces an increase in DAF protein levels on HMC, an effect that requires RNA and protein synthesis [15]. In those previous studies we also showed that ZAS and activation of C5 deficient serum have no significant effects on DAF protein. In contrast, we showed here that those incubations cause an increase in DAF mRNA. Overall, these observations suggest that although early complement components (activated C3 and C5a) cause an increase in DAF mRNA, terminal complement components may initiate a more complex signal that also causes an increase in DAF mRNA translation resulting in an increase in DAF levels on the cell membrane. It should also be noted that activation of terminal complement components causes a more long standing up-regulation of DAF mRNA levels than activation of early complement components, and perhaps these differences have an impact on DAF synthesis. The present study also demonstrates that DAF mRNA levels are increased after incubation of unstimulated HMC with protein synthesis inhibitors. These effects of cycloheximide may be due to an increase in mRNA half life [33], or by preventing the synthesis of an inhibitor of gene transcription [34]. Thus, it appears that in unstimulated HMC, DAF mRNA levels are maintained low by a process that requires protein synthesis.

The relative cytoprotective role of DAF and MCP varies among cells [11]. MCP levels on HMC are higher than DAF levels. However, as shown here, HMC can increase DAF levels, and presumably cytoprotection, when under complement attack. Further dissection of the signal transduction pathways that control DAF synthesis on HMC and other cells may suggest strategies that will permit us to modulate cytoprotection against complement effects. Because glomerular diseases are frequently associated with complement deposition, modulation of C'RP levels on glomerular cells may have beneficial effects in complement mediated diseases.

Acknowledgments

This work was supported by a National Institutes of Health grant, DK 433886. We thank A.P. Bakaletz for technical assistance and Ms. Elisabeth Smith for secretarial assistance.

Reprint requests to F.G. Cosio, M.D., Department of Internal Medicine, Nephrology, The Ohio State University, 1654 Upham Drive, Room N210, Columbus, Ohio 43210-1228, USA.

References

1. Lachman PJ: The control of homologous lysis. *Immunol Today* 12:311–314, 1991
2. Holers VM, Cole JL, Lublin DM, Seya T, Atkinson JP: Human C3b and C4b regulatory proteins: A new multi-gene family. *Immunol Today* 6:188–192, 1989
3. Fearon DT: Regulation by membrane sialic acid of β1H-dependent decay-dissociation of amplification C3 convertase of the alternative complement pathway. *Proc Natl Acad Sci USA* 75:1971–1975, 1978
4. Zalman LS, Wood LM, Muller-Eberhard HJ: Isolation of a human erythrocyte membrane protein capable of inhibiting expression of homologous complement transmembrane channels. *Proc Natl Acad Sci USA* 83:6975–6979, 1986
5. Rollins SA, Zhao J, Ninomiya H, Sims PJ: Inhibition of homologous complement by CD59 is mediated by a species-selective recognition conferred through binding to C8 within C5b-8 or C9 within C5b-9. *J Immunol* 146:2345–2351, 1991
6. Pangburn MK, Schreiber RD, Muller-Eberhard HJ: Deficiency of an erythrocyte membrane protein with complement regulatory activity in paroxysmal nocturnal hemoglobinuria. *Proc Natl Acad Sci USA* 80:5430–5434, 1983
7. Kinoshita T, Medof ME, Silber R, Nussenzweig V: Distribution of decay-accelerating factor in the peripheral blood of normal individuals and patients with paroxysmal nocturnal hemoglobinuria. *J Exp Med* 162:75–92, 1985
8. Holguin MH, Wilcox LA, Bernshaw NJ, Rosse WF, Parker CJ: Relationship between the membrane inhibitor of reactive lysis and the erythrocyte phenotypes of paroxysmal nocturnal hemoglobinuria. *J Clin Invest* 84:1387–1394, 1989
9. Holguin MH, Martin CB, Bernshaw N, Parker CJ: Analysis of the effects of activation of the alternative pathway of complement on erythrocytes with an isolated deficiency of decay accelerating factor. *J Immunol* 148:498–502, 1992
10. Medof ME, Gottlieb A, Kinoshita T, Hall S, Silber R, Nussenzweig V, Rosse WF: Relationship between decay-accelerating factor deficiency, diminished acetylcholinesterase activity and defective terminal complement pathway restriction in paroxysmal nocturnal hemoglobinuria erythrocytes. *J Clin Invest* 80:165–174, 1987
11. Matsumoto M, Sugita Y, Seya T: Alternative complement pathway mediated myeloid cell cytotoxicity: Repertoire of membrane factors participating in regulation of C3 deposition and cytolysis. *Eur J Immunol* 21:1787–1792, 1991
12. Kazatchkine MD, Fearon DT, Appay MD, Mandet C, Bariety J: Immunohistochemical study of the human glomerular C3b receptor in normal kidney and in seventy five cases of renal diseases. *J Clin Invest* 69:900–912, 1982
13. Cosio FG, Sedmak DD, Mahan JD, Nahman NS Jr: Localization of decay-accelerating factor in normal and diseased kidneys. *Kidney Int* 36:100–107, 1989
14. Shibata T, Cosio FG: Expression of cell-associated C3 regulatory proteins (CCRP) on human glomerular cells. *JASN* 1:538, 1990
15. Shibata T, Cosio FG, Birmingham DJ: Complement activation induces the expression of decay accelerating factor on human mesangial cells. *J Immunol* 147:3901–3908, 1991
16. Cosio FG, Sedmak DD, Nahman NS Jr: Localization and characterization of cellular receptors for matrix proteins in normal human kidney and human mesangial cells. *Kidney Int* 38:886–895, 1990
17. Chomczysnski P, Sacchi N: Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 162:156–159, 1987

18. NAHMAN NS JR, LEONHART K, COSIO FG, HEBERT CL: Effects of high glucose on cellular proliferation and fibronectin production by cultured human mesangial cells. *Kidney Int* 41:396–402, 1992
19. MEDOF ME, LUBLIN DM, HOLERS VM, AYERS DJ, GETTY RR, LEYKAM JF, ATKINSON JP, TYKOCINSKI ML: Cloning and characterization of cDNAs encoding the complete sequence of decay-accelerating factor of human complement. *Proc Natl Acad Sci USA* 84:2007–2011, 1987
20. LUBLIN DM, LISZEWSKI MK, POST TW, ARCE MA, LEBEAU MM, REBENTISC MB, LEMONS RS, SEYA T, ATKINSON JP: Molecular cloning and chromosomal localization of human membrane cofactor protein (MCP). Evidence for inclusion in the multigene family of complement regulatory proteins. *J Exp Med* 168:181–194, 1988
21. COSIO FG, SHEN X-P, BIRMINGHAM DJ, VAN AMAN ME, HEBERT LA: Evaluation of the mechanisms responsible for the reduction in erythrocyte complement receptors when immune complexes form in vivo in primates. *J Immunol* 145:4198–4206, 1990
22. HSI B-L, YEH C-JG, FENICHEL P, SAMSON M, GRIVAUX C: Monoclonal antibody GB24 recognizes a trophoblast-lymphocyte cross-reactive antigen. *Am J Reprod Immunol Microbiol* 18:21–25, 1988
23. CHO SW, OGLESBY TJ, HSI BL, ADAMS EM, ATKINSON JP: Characterization of 3 monoclonal antibodies to membrane co-factor protein (MCP) of the complement system and quantification of MCP by radioassay. *J Clin Exp Immunol* 83:257–261, 1991
24. CARAS IW, DAVITZ MA, RHEE L, WEDDELL G, MARTIN DW JR, NUSSENZWEIG V: Cloning of decay-accelerating factor suggests novel use of splicing to generate two proteins. *Nature* 325:545–549, 1987
25. MIZOGUCHI Y, TANIMOTO K, YUE C-L, HORIUCHI Y: Immune receptors (IgG-Fc and complement receptors) in normal human organs. *Lab Invest* 40:703–707, 1979
26. EDDY A, NEWMAN SL, COSIO FG, LEBIEN T, MICHAEL AF: The distribution of the CR3 receptor on human cells and tissue as revealed by a monoclonal antibody. *Clin Immunol Immunopath* 31:371–389, 1984
27. CARNEY DF, LANG TJ, SHIN ML: Multiple signal messengers generated by terminal complement complexes and their role in terminal complement complex elimination. *J Immunol* 145:623–629, 1990
28. SHENOY-SCARIA AM, KWONG J, FUJITA T, OLSZOWY MW, SHAW AS, LUBLIN DM: Signal transduction through decay-accelerating factor. Interaction of glycosyl-phosphatidylinositol anchor protein tyrosine kinases p56 and p59. *J Immunol* 149:3535–3541, 1992
29. MOUSLI M, HUGLI TE, LANDRY Y, BRONNER C: A mechanism of action for anaphylatoxin C3a stimulation of mast cells. *J Immunol* 148:2456–2461, 1992
30. SCHINDLER R, GELFAND JA, DINARELLO CA: Recombinant C5a stimulates transcription rather than translation of interleukin 1 (IL-1) and tumor necrosis factor: Translational signal provided by lipopolysaccharide or IL-1 itself. *Blood* 76:1631–1638, 1990
31. PELAYO JC, CHENOWETH DE, HUGLI TE: Effects of the analphylatoxin C5a on renal and glomerular hemodynamics in the rat. *Kidney Int* 30:62–67, 1986
32. SEKSE I, IVERSEN BM, DAHA MR, OFSTAD J: Acute effect of passive Heymann nephritis on renal blood flow and glomerular filtration rate in the rat: Role of the anaphylatoxin C5a and the alpha-adrenergic nervous system. *Nephron* 60:453–459, 1992
33. LAU LF, NATHANS D: Expression of a set of growth related immediate early genes in BALB 3t3 cells: Coordinate regulation with c-*fos* and c-*myc*. *Proc Natl Acad Sci USA* 84:1182–1186, 1987
34. AYANLARBATUMAN O, FERRERO AP, DIAZ D, JIMENEZ SA: Regulation of transforming growth factor beta 1 gene expression by glycocorticoids in normal human T lymphocytes. *J Clin Invest* 88:1574–1580, 1991